ROS: A Step Closer to Elucidating Their Role in the Etiology of Light-Induced Skin Disorders

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Free radicals are chemical species characterized by an odd number of orbital electrons, or by pairs of electrons of similar directional spin isolated singly in separate orbitals. Consequently, most of these agents are highly reactive and usually exhibit an extremely short half-life, although due to steric and resonance effects, some exceptions occur. For example, the diradical, molecular oxygen, exists in the triplet state ($^3O_2$) and is an essential element of normal metabolism of aerobic organisms. Even under normal circumstances, however, the controlled reduction of oxygen occurs and leads to the formation of the major reactive oxygen species (ROS), i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical. Singlet oxygen, although technically not a free radical, is highly reactive and may produce biological effects similar to those of other ROS. In addition to ROS, organisms may be subjected to a wide array of other free radicals, including those of both exogenous and endogenous origin. This is particularly true of the skin. Almost every major class of cellular chemical constituent (e.g., lipids, nucleic acids, and proteins) is subject to radical attack, providing a rationale for the involvement of free radical reactions in the pathophysiology of a wide spectrum of disorders. Although the idea that free radical-mediated reactions might be responsible for some of the deleterious effects observed in actinically exposed skin is now accepted as *fait accompli*, it was at first received with due skepticism. Certainly, it had been clearly demonstrated that radicals resulted after UV radiation of excised skin (Pathak and Stratton, 1968). But the reason for this caution was based upon the fact that methodology required for rigorous testing for involvement of free radicals in pathogenesis had not advanced to the stage that lent itself well to *in vivo* measurement (Black, 1987). Thus, the gradual acceptance of free radical involvement in specific cutaneous disorders came largely from a considerable body of circumstantial evidence based upon indirect lines of investigation involving the major participants in such reactions, i.e., pro-oxidants, free radicals, and anti-oxidants. Examples include the amelioration of symptoms or a disease state by antioxidants, or the measurement of lipid oxidation. With respect to the former, it is becoming clear that antioxidant function is more complex, producing physiological responses that may or may not result from free radical scavenging. In addition, there are a number of pathologies where lipid oxidation has been shown to increase with no concurrent exacerbation of disease development and sometime to occur concomitantly with amelioration of the disorder (Rhodes *et al*, 1995). Thus, the relevance of these indirect responses to the pathology of specific disorders remains unclear.

Recent advances in a number of analytical techniques, especially free radical spin trapping, have held promise to advance our knowledge of free radical roles in disease. Froncisz *et al* (1989) provided construction details, engineering characteristics, and spectroscopic performance data of a loop-gap resonator for the electron spin resonance (ESR) detection of spin labels. A low-frequency ESR spectrometer has been employed to measure free radical reactions in living mice (Utsumi *et al*, 1995). Free radical production in tissues of a living animal, the first report of such a direct measurement, employed low-frequency paramagnetic resonance, in combination with *in vivo* spin trapping, to detect hydroxyl markers produced from ionizing radiation in the tumor of a living mouse (Halpern *et al*, 1995). He *et al* (2001), using a specially designed bridged loop-gap surface resonator, described *in vivo* imaging of free radicals in human subjects with the use of topically applied nitroxide spin labels and ESR. Using various oxygen-sensitive nitroxide spin labels and ESR, it was found that trap intensity corresponded to irradiance and penetration of UVB and UVA in human skin biopsies (Herrling *et al*, 2003). In this issue of the *Journal*, Takeshita and colleagues, in a carefully controlled study, have employed the griseofulvin-induced protoporphyria mouse model and *in vivo* ESR spectrometry to examine ROS generation and their potential role in this photosensitive disorder (2004). Although porphyrias may exhibit an indeterminate pattern of inheritance, they represent a group of diseases that result from deficiencies in specific enzymes in heme biosynthesis that takes place in the erythropoietic system and liver (Cox, 1997). Accumulation of heme precursors leads to a distinct syndrome of cutaneous photosensitivity, thought to result partly from photodynamic reactions of ROS with porphyrins (Buettner and Oberley, 1980). Edema and erythema occur within a few minutes after light exposure. The griseofulvin-induced protoporphyria mouse model has been thoroughly documented and is employed as a standard model for the study of Erythropoietic protoporphyria (Konrad *et al*, 1975). If, indeed, ROS produce damage associated with the photodynamic

Abbreviations: DFO, desferr loxamine meystate; ESR, electron spin resonance; ROS, reactive oxygen species

reaction of protoporphyrin, then *in vivo* generation of these agents would have to be detected in the skin of griseofulvin-induced protoporphyria. This was approached by using L-band ESR spectrometry with a loop-gap resonator to measure the systemically administered probe (carbamoyl-PROXYL)[1] throughout a mouse, i.e., whole-body measurements. A surface resonator was then attached to the skin site to be examined, and ESR spectra were obtained. Comparison of ESR signal intensity after administration of the carbamoyl-PROXYL probe, and sensitivity mapping of the surface resonator, indicated that the ESR signal detected with the surface resonator came mainly from the probe distributed in the skin. The intensities of the low-field peaks were used to calculate signal decay. A good correlation between enhanced nitroxyl signal decay and ROS generation had previously been demonstrated (Miura *et al*, 1995). Measurements were taken in griseofulvin-fed mice and controls, before and after irradiation with a 300-W tungsten lamp, and after administration of saline control or ROS inhibitors. Light irradiation increased the signal decay rate (an indication of ROS formation) significantly in the griseofulvin-fed mice but had little effect on this parameter in control mice. Conversely, when the light was switched off, there was a rapid deceleration of the decay rate to the original level, i.e., the decay rate responded quickly and reversibly to light irradiation. Furthermore, superoxide dismutase, catalase, and the iron-chelating agent, DFO, when given intravenously prior to ESR measurement, suppressed the rate of signal decay in irradiated griseofulvin-fed mice in a concentration-dependent manner but had no effect on decay rate in non-irradiated mice.

Although speculation remains regarding the precise mechanism(s) of ROS action in this light-sensitive disorder, this is a report of *in vivo* detection of light-induced ROS in the skin of a living animal and makes an important contribution to fulfilling the criteria for acceptance of free radical participation in a disease state (Proctor and Reynolds, 1984) . The development of the surface resonator as an analytical tool for the detection of *in vivo* radical generation in the skin should be, as the authors note, extremely valuable in dermatology for examination of ROS generation, their possible role in a number of photosensitivity disorders, as well as in the evaluation of antioxidants and sunscreens in radical scavenging.

[1]3-carbamoyl-2,2,5,5-tetramethylpyrrolidine-*N*-oxyl, a stable nitroxide radical employed as a spin probe. It may also act as a superoxide dismutase (SOD) mimic compound. Thus, the increased decay rate of this redox probe after light exposure of skin in griseofulvin-fed mice supports the involvement of ROS species in mediation of symptoms in this photosensitive model.

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